

# Formation of Mutagens by Heating Foods and Model Systems

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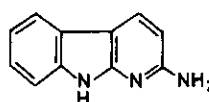
We have demonstrated that the pyrolysis products of amino acids and proteins in model systems are mutagenic. The mutagenic principles in the pyrolyzates of amino acids have been isolated and identified by Sugimura et al. We have isolated and identified amino- $\alpha$ -carbolines from pyrolyzed soybean globulin as mutagens. The yield of mutagens by the heating of food constituents is changed by the heating method. Effects of heating methods on the yield of amino- $\alpha$ -carbolines were studied in a series of experiments, and the results are shown in this paper. Additionally, it has been shown that by the heating of creatine-sugar mixtures imidazoquinoline or quinoxaline mutagens are formed.

We demonstrated previously that the pyrolysis products of amino acids and proteins in model systems are mutagenic (1,2). The mutagenic principles in the pyrolyzates of amino acids have been isolated and identified by Sugimura et al. (3,4). We have isolated and identified amino- $\alpha$ -carbolines from pyrolyzed soybean globulin as mutagens (5). The yield of mutagens by the heating of food constituents is changed by the heating method.

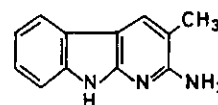
## Formation of Amino- $\alpha$ -carbolines

It was supposed that amino- $\alpha$ -carbolines (I) and amino- $\gamma$ -carbolines as well as Trp-P-1 or Trp-P-2 would be formed from the tryptophan moiety of protein. Therefore, a relationship between tryptophan content in the protein and the pyrolytic formation of mutagens was studied (6). Zein, gluten, globulin, casein, and albumin as well as chicken meat and horse mackerel were pyrolyzed at 550°C and the yield of mutagens was determined. The content of tryptophan in these materials was determined by an amino acid analyzer. The yield of amino- $\alpha$ -carboline correlated positively with the content of tryptophan in the protein, and it was 60 to 850 times that of Trp-P-2.

Yield of amino- $\alpha$ -carboline and Trp-P-2 per gram of tryptophan in the protein was calculated (Table 1). The yield of amino- $\alpha$ -carboline per gram of tryptophan in the protein was higher than that of free tryptophan, especially for albumin and chicken meat. Although proteins and proteinaceous materials tested in this experiment showed appreciably higher yield of amino- $\alpha$ -carboline than that expected from the tryptophan content,



(I)



(II)

the yield of Trp-P-2 per gram of tryptophan in the protein was lower than that of free tryptophan and indicated little difference among the proteins tested. The reason for the formation of higher amounts of amino- $\alpha$ -carboline than expected from the tryptophan contents remains obscure.

The effect of heating temperature on the yield of amino- $\alpha$ -carboline was determined using albumin. Amino- $\alpha$ -carboline was formed when albumin was heated to a temperature of 200°C, and the concentration increased with increasing temperature. The maximum yield of the carboline was obtained at 600 to 700°C, and the ratio of amino- $\alpha$ -carboline to mutagenic activity of whole heated product was approximately constant among the heating temperatures.

The effect of gases above heated albumin on the yield of amino- $\alpha$ -carboline was determined. A higher yield was obtained by heating albumin under a N<sub>2</sub> atmosphere than by heating under air. It was difficult to detect Trp-P-2 below 300°C, even if albumin was heated under N<sub>2</sub> (7).

Modification of pyrolytic formation of amino- $\alpha$ -carboline was tested using some antioxidants. Quercetin, tannic acid, *n*-propyl gallate, catechin, BHT, and rutin were added to albumin, and the yield of amino- $\alpha$ -carboline was determined after heating at 550°C. The yield

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Table 1. Yield of amino- $\alpha$ -carboline and Trp-P-2 in the pyrolyzate of protein systems.

Protein	Yield, $\mu\text{g/g}$ protein		Tryptophan content, %	Yield, $\mu\text{g/g}$ tryptophan	
	Amino- $\alpha$ -carboline	Trp-P-2		Amino- $\alpha$ -carboline	Trp-P-2
Zein	0.05	0	0	—	—
Gluten	1.2	trace	0	705	—
Globulin	1.5	0.01	0.20	750	5
Casein	3.8	0.06	0.93	409	7
Albumin	43	0.06	1.02	4216	6
Chicken meat	34	0.04	0.76	4474	5
Horse mackerel	12	0.02	0.69	1139	3
Tryptophan	—	—	—	250	10

Table 2. Contents of amino- $\alpha$ -carbolines in grilled foods.

Grilled food	Amino- $\alpha$ -carboline, ng/g	Me-amino- $\alpha$ -carboline, ng/g
Beef	651	64
Chicken	180	15
Chinese mushroom	47	5
Onion	2	ND

of amino- $\alpha$ -carboline was effectively decreased by the addition of these antioxidants (8).

## Amino- $\alpha$ -carbolines in Grilled Foods

The amount of amino- $\alpha$ -carboline and methylamino- $\alpha$ -carboline (II) in grilled foods was determined (Table 2). Because the yield of amino- $\alpha$ -carbolines in the pyrolyzed proteinaceous materials was higher than the levels of Trp-P-1 and Trp-P-2 as well as imidazoquinoline or quinoxaline mutagens, the analysis of amino- $\alpha$ -carboline is comparatively easy. If the cooked food is purified for analysis of amino- $\alpha$ -carbolines, the recovery of amino- $\alpha$ -carboline is decreased markedly. Therefore, we have used a simple method for the determination of amino- $\alpha$ -carbolines in the cooked food. A methanol extract of the sample was subjected to Sephadex LH-20 column chromatography, and the fractions corresponding to amino- $\alpha$ -carbolines were applied to an HPLC column. Amino- $\alpha$ -carbolines were detected by a fluorescence light detector.

The contents of amino- $\alpha$ -carbolines in grilled foods are shown in Table 2. Beef, chicken, chinese mushrooms and onions were grilled for 6 to 15 minutes on a grill placed on a gas stove with 3-cm high flame. The sample was turned often to grill both sides equally; 651 ng of amino- $\alpha$ -carboline per gram of grilled beef was detected. A study has shown that 50 ng of Trp-P-2 was present per gram of grilled meat (10). Thus, the content of amino- $\alpha$ -carboline in the grilled meat was higher than that of Trp-P-2.

## Mutagen Formation from Creatine

On the outer part of the surface of cooking fish or meat, temperatures may often rise to 300°C and thus mutagens such as amino- $\alpha$ -carbolines may be formed.

Table 3. Mutagen formation from creatine at 200°C.

Substances	His <sup>+</sup> revertants/g creatine
Creatine + glucose	$19.2 \times 10^4$
Creatine + oleic acid	$8.0 \times 10^4$
Creatine + 17 amino acid mixture	$111.1 \times 10^4$
Creatine	$0.1 \times 10^4$
Glucose (1 g)	0
Oleic acid (1 g)	0
17 Amino acid mixture (1 g)	$4.9 \times 10^4$

In the food, amino acids and proteins exist together with the other compounds. At cooking temperatures below 200°C, the mutagen may be formed by the reaction of nitrogenous compounds with sugar or fatty acid in the fish and meat. The mixtures of nitrogenous compounds and glucose, fatty acids or quinones were heated and the basic fraction of the product was used for mutagenicity assay.

The mixtures of nitrogenous compounds and glucose were heated by refluxing at 100°C for 8 hr, and the mutagenicity of the products was determined. No or extremely low mutagenicity was detected when albumin, adenine, or amino acid mixture was refluxed with the addition of glucose. However, the mixture of creatine and glucose showed appreciable mutagenicity when it was refluxed. Mutagens in beef extract may be formed by the reaction of creatine and glucose during the preparation (11).

Refluxing of individual amino acids in the presence of glucose was also carried out. Significant mutagenic activity was not detected by heating of amino acids except arginine and lysine. From lysine, mutagens susceptible to both TA 98 and TA 100 were formed. With arginine, mutagenic activity was shown at approximately the same level as with creatine (11). On the cooking of meat, the temperature of the surface sometimes reaches 150°C or more. So, the mixtures of equal weight of nitrogenous compounds and glucose were heated in the oven at 150°C for 1 hr, and the mutagenic activity of the product was determined. No mutagenicity was detected by heating the mixture of albumin and glucose, and low mutagenicity was detected in the heated amino acid mixture and glucose (Table 3). Mutagenic activity of the products of a heated creatine and glucose mixture was remarkably high. Mutagenicity was not detected in the heated prod-

ucts of creatine or glucose alone (12).

Creatine, amino acid mixture, adenine, or albumin were heated at 200°C with or without addition of oleic acid, and the mutagenicity of the mixtures was determined (Table 3). Higher mutagenicity was obtained by heating the mixture of creatine and oleic acid. Heating the amino acid mixture also produced mutagenicity, which was enhanced by the addition of oleic acid (13).

The effect of heating temperature on the mutagenicity of heated creatine and oleic acid mixture was examined. A detectable degree of mutagenicity was formed at 100°C, and the mutagenic activity was increased by raising the heating temperature. The temperature of oil in frying is around 180°C, so mutagens would be formed from the reaction of creatine with fatty acids during frying (19).

In fish and meat, some of the amino acid is present in the free form. On the heating of meats at cooking temperature, mutagen formation by the interaction among the different kinds of nitrogenous compounds would be possible. Mixtures of the 17 amino acids were heated at 200°C with or without addition of creatine (Table 3). With the addition of creatine, the mutagenic activity of heated amino acid mixture was increased remarkably. It is clear that creatine showed higher mutagenic activity when it was heated with the amino acid mixture than when it was heated with glucose or oleic acid.

Mutagenicity of heated mixtures of individual amino acid and creatine was determined. No or little mutagenicity was detected in the heated products of amino acid or creatine alone at 200°C. Remarkably high mutagenicity was detected in the heated products of cystine, threonine, phenylalanine, methionine, tryptophan, valine, proline, or serine with creatine. Free cystine or tryptophan is not contained in the meat; therefore, mutagen formation by heating creatine and cystine or tryptophan would not be expected during cooking of meat. It was concluded that the mutagens would be formed from threonine, phenylalanine, methionine, valine, and proline with creatine during the cooking of meat (14).

Kasai et al. isolated aminoimidazole compounds from the cooked fish and meat as mutagenic principles (15,16). It was expected that the mutagenic principles in the heating products of creatine and glucose, fatty acid or amino acids might be imidazole compounds. We have tried to isolate 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) from the heated creatine and glucose mixture or creatine and oleic acid mixture. But no IQ was detected in the heated products of these mixtures. Mixtures of creatine with cystine, threonine, phenylalanine, methionine, tryptophan, valine, proline, or serine were heated, and IQ in the products was separated by liquid-liquid partitioning, thin layer chromatography, and high-pressure liquid chromatography. No IQ was detected in the mixtures of creatine with cystine, threonine, phenylalanine, methionine, tryptophan or valine. In the mixture of creatine and proline, IQ was isolated and identified by mass spectra, UV spectra and mutagenic activity (17).

It is known that proline as well as creatine are common components in fish and meat. Therefore, it would be probable that IQ is formed from the reaction of creatine with proline during cooking. Ashoor et al. (18) reported that, of 20 common amino acids added individually to ground beef patties prior to frying, only proline enhanced the formation of mutagenic activity. The mechanism of the formation of IQ by heating the mixture of creatine and proline remains obscure. Formation of IQ in the model system consisting of creatine, glycine, and glucose proposed by Jägerstadt et al. (19) was also tested, but no IQ was detected in the product of model system.

Heating of organic compounds causes the formation of various kinds of free radicals, and the mutagens may be formed by the reaction among the radicals during heating. Quinones are known to be radicals when they are oxidized, and they are contained in the biological materials and the pyrolyzate. It is probable that the mutagens are formed by the radical reaction between quinones and amino acid during cooking. We determined mutagenicity of the heated products of the mixtures of hydroquinone and amino acid. Amino acid was heated with or without addition of hydroquinone at 250°C for 1 hr under air. Without hydroquinone, no or low mutagenicity was detected in the heated products. However, with the addition of hydroquinone, mutagenic activity was detected in the heated products of amino acid, except glutamic acid or aspartic acid. Higher mutagenic activity was detected by heating ornithine, threonine, methionine, tryptophan, lysine, arginine, or alanine with hydroquinone (20). The products of heating some of the amino acid-hydroquinone mixtures at 250°C yielded  $10^6$  or more revertant colonies per mmole with *Salmonella*. This level of mutagenicity is similar to that obtained by pyrolysis of tryptophan at 600°C. Identification of the mutagens in the heated amino acid-hydroquinone mixtures is in progress.

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